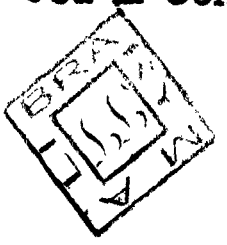
Qualitative and Quantitative Histochemical Studies on *Avitellina lahorea*, Woodland, 1927

DISSERTATION SUBMITTED
IN PARTIAL FULFILMENT FOR THE DEGREE OF
MASTER OF PHILOSOPHY
IN
ZOOLOGY (Parasitology)



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Certified that the work embodied in this dissertation is original and has been done under my supervision by Mr. Rashid Farooq. I consider it suitable for submission towards partial fulfilment of the requirements of the degree of Master of Philosophy (M.Phil) in Zoology of the Aligarh Muslim University, Aligarh.

(Hisamuddin Farooqi)

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Rashid Farooq

(RASHID FAROOQ)

INTRODUCTION

Avitellina lahorea, Woodland, 1927 is a common and important parasite of sheep and goats in the agrarian tropics and subtropics. Its high incidence and relatively large number in infected hosts obviously effects the health of livestock and thus entails considerable economic importance.

In a random sampling carried out at Aligarh from August 1977 to July 1978 fully grown worms were found in the intestines of sheep and goats. The average worm burden was found to be 105 % (Table-I). Usually this parasite was found alongwith Moniezia expansa and Stilesia globipunctata concurrently. All the three inhabit the same host and occupy the same ecological niche and appears to maintain a dynamic equilibrium in the parasitic population of the host. This provides a good opportunity to find out the factors relating to concurrency of infection of these closely related parasite species, viz., M. expansa, A. lahorea and S. globipunctata in the definitive host.

The absence of infection may be attributed as a result of seasonal variation. From May/June to September poor infection rate was noticed and the worms thus found were rather small and emaciated.

The life cycle of A. lahorea has not yet been elaborated but keeping in view the life cycle pattern of other anoplocephalids like

M.expansa, (vide Stunkard, 1937) and A.centripunctata (vide Narāspur, 1974) it can be presumed that the intermediate host of A.lahorea is either an oribatid mite or a velvet mite. These mites abound during the post-mons on period when the biotope offers better sustaining facilities to these acarines. The worms obtained during the post-monscon period and for a few further months were rather small and this indicated that the infection has settled only lately.

Sheep and goats bought at the Aligarh abbatoir were mostly from the adjoining areas. They grazed extensively in the outskirts and were therefore subjected to a higher frequency of contacting the inf -ction. Relatively high worm-burden was found from January to May but from May to September the infection was almost negligible; from September to January the inf-ction was heavy but the worms were very small which indicated that the hosts acquired infection during the post-monsoon period. However, what seems more important from the pathobiological point of view is the worm-burden rather than the number of animals found infected in a sample.

Although A.lahorea is quite an important parasite from the agricultural and economic points of view, little work seems to have been done on these species, except for the comprehensive anatomical study of Gough (1911) on A.centripunctata and of

Martinez - Gomez (1966) on morphological and chromatographic investigations on cestodes of the family Anoplocephalidae. However, not much morphological information is available about A.lahorea, although the literature on physiology and biochemistry of M.expansa and M.benedeni is rather extensive.

The relatively high and concurrent incidents and easy availability of A.lahorea provided a good opportunity for detailed study and investigations regarding the physiology and morphology of the parasite and in view of the facility, preliminary histochemical and biochemical studies accorded priorty.

In the present study, preliminary histochemical and biochemical investigations relating to scolex, neck, immature, mature and gravid proglottides of A.lahorea has been carried out, whereas studies on other aspects will follow.

TABLE I. Incidence of *A. lahorea* infection at Aligarh based on a fortnightly random sampling.

Month/Year	No. of hosts examined.	No. of infected hosts	% of infection	Maximum worm burden	Mean intensity of infection (No. per infected host)
Aug. 1977	96	5	5.20	5	1.00
Sept.	110	6	5.45	2	0.33
Oct.	99	25	25.252	157	6.28
Nov.	101	23	22.772	174	7.56
Dec.	108	43	39.814	209	4.86
Jan. 1978	105	33	31.428	213	6.45
Feb.	97	41	42.268	186	4.53
March.	95	28	29.473	197	7.03
April.	109	27	24.77	135	5.0
May.	103	7	6.796	23	3.28
June.	110	0	0	0	0
July.	107	7	8.41	7	0.77

Average worm burden 1.05 per host.

HISTORICAL REVIEW

The genus Avitellina was erected by Gough (1911) with A. centripunctata (Rivolta, 1874) as its type species. Rivoltas' original description for A. centripunctata from Italy was rather inadequate and inconclusive later on it was supplemented by studies of Gough (1911), based on material from sheep of South Africa. Later on Woodland (1927) in a comprehensive review concluded that what Gough (1911) has described as A. centripunctata was a composite species and contained more than one. Based on the shape of the paruterine organ he splitted A. centripunctata (sensu Gough, 1911) into two, namely A. centripunctata (Rivolta, 1874) sensu stricto and A. goughi which was characterised by pyriform and snail shaped paruterine organs.

Woodland (1927) examined more critically the material pertaining to various species of the genus Avitellina from different localities and in addition to A. goughi he added three more specie namely A. sudanea (Woodland, 1927) from sheep of Khartoom in Sudan; A. lahorea (Woodland, 1927) from sheep in Lahore and A. chalmersi (Woodland, 1927) from sheep in Khor Barboi, North Africa. Woodland's descriptions were, however, sketchy because the materials mostly comp rised fragments of strobila. On what he based A. lahorea were two pieces of strobila, one immature

and the other male mature and similar was the case with the other species he descriped. The original descriptions of these species, in fact had much to be desired for the provision of complete description. In none of the cases the scolex was found and therefore, much room was left for critical systematic evaluation of the genus Avitellina andits constituent species. Southwell (1930) reported only three species of this genus from India, namely, A. centripunctata, A. goughi and A. lahorea. This author based the classification of the species on the number and position of testes vis-a-vis the outer osmoregulatory canal and also the shape of the paruterine organs. Subsequently, a more comprehensive evaluation and revision of the genus Avitellina from India was made by Bhalerao (1936) and his study was based on substantial material obtained from various localities. In the study under review Bhalerao (1936) recognized ten distinct species, namely, A. woodlandi (Bhalerao, 1936) and A. tatia (Bhalerao, 1936), and eight previously known forms, namely, A.pintneri, A. sudanea, A.lahorea, A.goughi, A.southwelli, A.chalmersi and A.centripunctata Bhalerao's (1936) descriptions, were more precise because they were made from a good collection of specimens, each provided with a scolex. Earlier Woodland (1935) had expressed doubt regarding the occurence of A. centripunctata or A.goughi in India, but

Bhalerao upheld and recognized the observations of Meggit (1927) and Southwell (1930) regarding the occurence of A.centripunctata in India. Even Yamaguti (1959) is of the opinion that A.centripunctata is a predominantly European species, and is not found in India. Lopez Neyra (1946) has also reviewed the genus and has lumped quite a few species under A.centripunctata although this view has not been upheld by subsequent investigators (Spassky, 1951).

As regards the systematic position and faunistics of the Indian species of the genus Avitellina, the present writer fully endorses the views and descriptions as given by Bhalerao (1936) and considers his views more authentic.

The description of A.lahorea (Woodland, 1927) as reviewed by Bhalerao (1936) is highly conclusive and convincing. He has based his description on ample material obtained from goats in Nagpur. Contrary to the description given by Woodland, (1927), based on only two fragments of strobila, the one furnished by Bhalerao (1936) was based on entire worms with intact scolex. He has also described certain intraspecific variations which have helped subsequent authors in ineffectively ascertaining the specific identity of A.lahorea (Woodland, 1927) and its other close Indian congeners.

Since the publication of Bhalerao's investigation in 1936, no significant contribution appears to have been made towards the

biology or systematics of the species of the genus Avitellina in India, except those of Naraspur (1974a) on the life cycle of A.lahorea and Naraspur (1974b) on certain ecological studies on the oribatid fauna of the Bombay region in relationship with the life cycle patterns of common anoplocephalid tapeworms including A.lahorea.

Although substantial work has been done on the morphology, physiology and biology of M.expansa, viz., Tower (1900); Osterlin and von Brand (1933); Stoll (1937); Wardle(1937); Yamao (1952); Verma (1956); Erasmus (1957); Singh and Singh (1958); Logachev and Dimitrova (1961); Howells (1965, 1969); Cheah (1967); Davidov (1969); Howells and Erasmus (1969) ; and Naraspur (1976); not much work has been done in regard to the other two most important genera namely Avitellina and Stilesia whose species occur concurrently with M.expansa in India and elsewhere as well. By far, the only comprehensive study on the morphology of A.centripunctata is that of Gough (1911) and of Martinez-Gomez (1966) on the amino acid contents of three species, M.expansa, A.centripunctata and S.globipunctata. So far the only study made on S.globipunctata is that of Gupta and Khera (1970) which relates to tegument, musculature and scolex anatomy and Amjadi (1971) which relates to its histopathology of infection.

Other physiological aspects of the species of the genus Avitellina and Stilesia except the ones mentioned above have not

at all been studied and offer good opportunity for investigations. The present study is a preliminary account of studies of histo-chemical & physiological studies planned and endevoured in regard to A.lahorea Woodland, 1927. which occurs quite commonly and concurrently with M.expansa and S.globip nctata in the ovine and caprine hosts in India.

6. Acetone Sudan black for bound lipids.

7. Indoxyl acetate for non-specific esterases.

Tests 1-6 were carried out in sections but test 7 was done in toto.

The staining procedures were in accordance with those cited in Lillie (1954); The BDH Manual (1972); and Pearse (1975).

Kodacolor II CI 35-36, ASA 80 and 20 Din and Fortepan (minature rapid film) ASA 160 and 23 Din were used for colour and black and white photography respectively.

The follwing quantitative estimations were made so as to substanciate the histochemical tests;

I. <u>For glycogen:</u> (Table III)

The extracts were made according to the method of Ashman and Seed (1973) and determined spectrophotometrically on Spectronic-20 through Montgomery's (1957) method.

II. <u>For Proteins:</u> (Table IV)

Estimations on wet tissue samples were made according to the method of Lowry and Rosenbrough (1951).

III. <u>For Lipids:</u> (Table V)

For the quantitative estimations of lipids, the conventional method of Kjehldahl soxhlet extraction through petroleum ether was preferred

over other solvents like ethanol-ether because the latter dissolves substances other than lipids as well. Ten samples of worms of known weight (2.5 grams each) were first oven dried and then subjected to extraction.

OBSERVATIONS

HISTOCHEMICAL

Tegument:

The cuticle appeared strongly positive (+++) to PAS (PlatesIIa, IVa) but negative (-) to Best's carmine (Plate IIIb). It stained moderately (++) with Alcian blue. Mercury-bromophenol blue gave a strong reaction (+++). Sudan black B and Acetone Sudan black gave negative (-) reactions (Plate VIa,b.).

The subcuticle was found to be moderately positive (++) to PAS (PlatesIIa, IVa) as well as to Best's carmine (PlateIIIb), but gave a negative reaction (-) with Alcian blue. It was strongly positive (+++) to Mercury-bromophenol blue. Sudan black B and Acetone Sudan black gave moderate (+ +) reactions (Plate VIa,b).

Musculature:

The general body muscles gave strong (+++) reactions with PAS (PlatesIb,IIa), Best's carmine (Plate IIIa) and Mercury-bromophenol blue. These however, indicated slight (+) response to Sudan black B and Acetone Sudan black (Plate Va,b), but gave a negative (-) reaction with Alcian blue.

Parenchyma:

The parenchyma appeared strongly positive (+++) to PAS (Plate IIa,b); Best's carmine (Plate IIIb); and Mercury-broncphenol blue but was negative (-) to Alcian blue. With

Sudan black B and Acetone Sudan black it gave a very faint reaction (+) (Plate VIa,b).

Suckers:

Suckers particularly the intrinsic muscle fibres gave an intensely positive (+++) reactions with PAS (Plates Ib, IIa); Best's carmine (Plate IIIa) and Mercury-bromophenol blue, but a negative (-) response was indicated with Alcian blue. The reactions with Sudan black B and Acetone Sudan black were quite faint (+) (Plate Va).

Testes and the Ovary:

The reactions with PAS, Best's carmine and Mercury-bromophenol blue were intensely positive (+++), whereas, Alcian blue, Sudan black B and Acetone Sudan black gave negative (-) reactions.

The Uterus and the Cirrus sac:

The uterus and the cirrus sac gave intense (+++) reactions with PAS, Best's carmine and Mercury-bromophenol blue. These gave negative (-) reaction with Alcian blue but slightly positive (+) one with Sudan black B and Acetone Sudan black. Additionally, the cirrus sac gave a strongly positive (+++) reaction with Indoxyl acetate. (Plate IX).

Sections of various regions were also partially and fully predigested with α- amylase but these sections either gave very faint reactions or completely failed to give any reaction with PAS and Best's carmine (Plate IVb).

Excretory ducts:

The excretory ducts failed to give any reaction with the conventional methods except for a slight (+) reaction with Sudan black B, indicating the assimilation of fats through their luminal walls.

The Nervous System:

The nervous system failed to give any reaction with the conventional methods, but test with Indoxyl acetate gave strongly positive (+++) reaction (Plates VII-X). The test with Indoxyl acetate was performed in toto and not in sections.

The results of various histochemical tests are summarized in Table II.

TABLE II. Results of various histochemical tests performed on A.lahorea.

S.No.	Substance investigated.	Histochemical test performed	Tegument		Musculature	Parenchyma	Suckers	Testes / Ovary	Uterus / Cirrus Sac	Excretory Ducts	Nervous system
			Cuticle	Subcuticle							
1.	Glycogen	Best's carmine	-	++	+++	+++	+++	+++	+++	-	-
2.	Glycogen & other Polysaccharides	PAS	+++	++	+++	+++	+++	+++	+++	-	-
3.	Acid mucopolysaccharides	Alcian blue	++	-	-	-	-	-	-	-	-
4.	Proteins	Mercury bromophenol blue	+++	+++	+++	+++	+++	+++	+++	-	-
5.	Bound lipids	Acetone Sudan black	-	++	+	+	+	-	+	-	-
6.	Lipids	Sudan black B	-	++	+	+	-	-	+	+	-
7.	Non-specific esterases	Indoxyl acetate	-	-	-	-	-	-	+++ (Cirrus sac)	-	+++

Intensely stained = +++
Moderately stained = ++
Slightly stained = +
Negative = -

OBSERVATIONS

BIOCHEMICAL:

GLYCOGEN: (Table III)

The total glycogen estimated in fresh tissue was found to be 0.981 (± 0.01) mg/gm wet tissue.

PROTEIN: (Table IV)

The total protein estimated in the fresh tissue was found to be 1.405 (± .021) mg/gm wet tissue.

LIPIDS: (Table V)

The total lipids estimated in fresh tissue was found to be 3.306 (± 0.0911) % of wet tissue.

TABLE III. Estimation of Total Glycogen in fresh tissue of A.lahorea.

No.of observations	Percentage transmittance	O.D.	Formula x 8500	d	d^2
1.	74	0.113	960.5	20.825	433.68062
2.	76	0.119	1011.5	30.175	910.53062
3.	74	0.113	960.5	20.825	433.68062
4.	75	0.125	1062.5	81.175	6589.3806
5.	78	0.108	918.0	63.325	4010.0556
6.	77	0.114	969.0	12.325	151.90562
7.	76	0.119	1011.5	30.175	910.53062
8.	74	0.113	960.5	20.825	433.68062
9.	76	0.119	1011.5	30.175	910.53062
10.	75	0.125	1062.5	81.175	6589.3806
11.	77	0.114	969.0	12.325	151.90562
12.	78	0.108	918.0	63.325	4010.0556
13.	77	0.114	969.0	12.325	151.90562
14.	76	0.119	1011.5	30.175	910.53062
15.	76	0.119	1011.5	30.175	910.53062
16.	78	0.108	918.0	63.325	4010.0556
17.	74	0.113	960.5	20.825	433.68062
18.	74	0.113	960.5	20.825	433.68062
19.	75	0.125	1062.5	81.175	6589.3806
20.	78	0.108	918.5	63.325	4010.0556
			Total = 19626.5		Total = 42985.13726

$$S.D. = \sqrt{\frac{\Sigma d^2}{N-1}}$$

$$= \sqrt{\frac{42985.13726}{20 - 1}}$$

$$= \sqrt{2262.375645}$$

$$= 47.564436$$

$$S.E. = \frac{S.D.}{\sqrt{20}}$$

$$= \frac{47.564436}{\sqrt{20}}$$

$$= \frac{47.564436}{4.4721359}$$

$$= 10.635731$$

The Total Glycogen in fresh tissue of A.lahorea was found to be 0.981 ($\pm$ 0.01) mg/gm.

TABLE IV. Estimation of Total Protein in fresh tissue of A.lahorea.

No. of observations	Percentage transmittance	O.D.	Formula x 8500	d	d^2
1.	67.5	0.171	1453.5	47.6	2265.76
2.	68.0	0.168	1428.0	22.1	488.41
3.	69.0	0.161	1368.5	37.4	1398.76
4.	70.5	0.149	1266.5	139.4	19432.36
5.	69.5	0.158	1343.0	62.9	3956.41
6.	68.0	0.168	1428.0	22.1	488.41
7.	66.5	0.177	1504.5	98.6	9721.96
8.	68.5	0.164	1394.0	11.9	141.61
9.	67.0	0.174	1479.0	73.1	5343.61
10.	68.5	0.164	1394.0	11.9	141.61
		Total =	14059.0	Total =	43378.9
		Mean =	1405.9		

$$S.D. = \sqrt{\frac{\Sigma d^2}{N-1}}$$

$$= \sqrt{\frac{43378.9}{9}}$$

$$= \sqrt{4819.8777}$$

$$= 69.425339$$

$$S.E. = \frac{S.D.}{\sqrt{10}}$$

$$= \frac{69.425339}{3.1622776}$$

$$= 21.95422$$

The Total Protein estimated in the fresh tissue of A. lahorea was found to be 1.405 (± 0.021) mg/gm.

TABLE V. Estimation of Total Fat in fresh tissue of A.lahorea.

No.of observations.	gm fat/2.5 gam each fresh tissue	gm fat/100 gms fresh tissue'x'	difference of mean and 'x'(d)	d^2
1.	0.0686	2.744	0.5620	0.315844
2.	0.0773	3.092	0.2140	0.045796
3.	0.0835	3.340	0.0340	0.001156
4.	0.0868	3.472	0.1660	0.027556
5.	0.0786	3.144	0.1620	0.026244
6.	0.0837	3.348	0.0420	0.01764
7.	0.0697	3.788	0.4820	0.232324
8.	0.0789	3.156	0.1500	0.022500
9.	0.0856	3.424	0.1180	0.013924
10.	0.0858	3.552	0.2460	0.066516
		Mean 'x'= 3.3060		Σd^2 = 0.747624

$d^2 = 0.747624$

Mean 'x' = 3.3060

$$S.D. = \sqrt{\frac{0.747624}{9}} = 0.2882175$$

$$S.E. = \sqrt{\frac{0.2882175}{10}} = 0.0911423$$

The result of ten analysis show that the fat content of A. lahorea ranges from 2.744 to 3.788 % with an average of 3.306 ± 0.0911 of fresh tissue weight.

DISCUSSION

HISTOCHEMICAL STUDIES

Tegument: There is convincing evidence that the topical layer of the cestode tegument is a uniform coat of muco-polysaccharides in order to overcome the enzymatic action of the host's intestinal secretions (Monné, 1959). These muco-polysaccharides mostly comprise acid glycans with oligo or polysaccharide moieties of glycoproteins and glycolipids. The glycans are composed of neutral hexoses, amino sugars and some esters and all these constitute a complex of polysaccharide which is not only antigenetically potent but may also act as an inhibitor of peristaltic activity of the intestine.

In the present study this topical layer is referred to as the comidian layer (Gough, 1911) has indicated a markedly positive (++) reaction with Alcian blue though it fails to give a reaction in any other part of the body, because it is an enzyme inhibitor and acid muco-polysaccharide forms a protective covering around the parasite so as to overcome the enzymatic action of the host's intestinal mucosa. The cuticle has been found to be strongly PAS positive (+++) (Plates IIa, IVa) which is an indication for the presence of glycocalyx, a conjugated carbohydrate other than glycogen. However, this region was found to be free of glycogen, thus giving a negative (-) reaction to Best's carmine test(Plate IIIb). It gives a strongly positive reaction (+++) with Mercury bromophenol blue which indicates a high protein percentage. Sudan black B and Acetone Sudan black failed to give any reaction (-) indicating

the absence of fats and bound lipids.

The next one or the homogenous layer (sensu Gough, 1911) and the basement membrance show similar reactions. The two layers constitute the major metabolic pathway of the cestode tegument. The homogenous layer which is thicker and presumably the site of major enzymatic activities contains the phosphatase system besides other enzymes mainly localized in the tegument syncitium as evidenced in M. expansa (Erasmus, 1957).

The subcuticle on the other hand is metabolically active and comprises the syncytium and the subcuticular cells. It gives distinctly positive (+++) reactions with PAS (Plates IIa, IVa) and Best's carmine (Plate IIIb) tests, thus indicating the sites of glycogen assimilations.

Further tests on sections partially and wholly digested with α-amylase revealed the partial or complete absence of glycogen, since sections predigested with α-amylase give very faint or failed to give any reaction (Plate IVb).

Tests with Mercury bromophenol blue revealed their high proteinaceous nature, since it gives a strongly positive (+++) reaction. Some bound lipids were found in the basal layer which revealed the nature of this layer as a connective tissue. It gives a moderately positive (++) reaction with Acetone Sudan black, but free fat was completely absent as Sudan black B failed to give any reaction.

Musculature : Like other muscular organs such as the suckers, the cirrus sac and the uterus, the results obtained in this case were

almost identical except for the presence of fat and bound lipids which are in appreciable quantities (++).

The glycogen and protein, however, appears in appreciable quantity. The high quantity of glycogen as revealed by strong positive reactions (+++) with PAS (Plates Ib,IIa) and Best's carmine (Plate IIIa) indicate frequent muscular movements for which the energy is supplied by this muscular glycogen, and a strong positive reaction (+++) with Mercury bromophenol blue suggests its proteinaceous nature.

Parenchyma : The parenchyma gives a very strong reaction (+++) with PAS (Plate IIa,b) and Best's carmine (Plate IIIb) but gives a negative (-) response in sections fully digested with α-amylase and slight reaction where the digestion was incomplete (Plate IVb). The result ascertains that glycogen is present in appreciable quantity in the parenchymal cells and confirms its character of a principal glycogen storing system. These facts further indicate the route of nutrient assimilation i.e., through the homogenous layers, the parikerya, the subcuticular cells and are thus stored in the cytoplasm of the parenchymal cells.

Mercury bromophenol blue gives a very strong reaction (+++) in the parenchyma, thus indicating a high protein level in the parenchymal cell walls.

A faintly positive (+) reaction with Acetone Sudan black indicates the presence of bound lipids within the parenchymal cells (Plate VIa,b). These bound lipids may be glycolipids, phospholipids or lipoproteins. The staining reaction with Sudan black B is also rather faint (+) which indicates a very low fat percentage.

Suckers : In suckers the intrinsic fibres give intense positive reaction (+++) to glycogenophilic stains like PAS (Plates Ib,IIb) and Best's carmine (Plate IIIa). In sections predigested with α-amylase, PAS and Best's carmine fail to give intense reactions (Plate IVb), thus confirming the presence of glycogen because it serves as a stored energetic material, the need of which can be revealed by the muscular movements which are brought about in order to maintain themselves within the host body. The reaction with Mercury bromophenol blue is intense (+++) which indicates a high protein level. The reactions with Sudan black B and Acetone Sudan black are rather light (+) which indicated the presence of fats and bound lipids (Plate Va) in relatively smaller quantities. The presence of lipids in such low quantities shows that the region is almost fat free.

The Ovary and the Testes : The results obtained for the ovary and the testes were identical so both are discussed together.

The ovary and the testes are most important sexual organs in the sense that the former produces the ova and the later produces the sperms. Since these two are produced in very large numbers, the energy requirement in the form of glycogen is also very large. The tests with PAS and Best's carmine give an intensely positive (+++) reaction. This fact is further proved when sections predigested with α-amylase fail to give any reaction.

They give intensely positive reaction (+++) with Mercury bromophenol blue, indicating a high protein level because in the

formation and liberation of sperms and eggs more and more protein is needed.

Further tests show that these two are devoid of fats and bound lipids, since Sudan black B and Acetone Sudan black fail to give any reactions.

The Cirrus sac and the Uterus : The cirrus sac and the uterus are highly muscularised organs. Since both have to perform much muscular activities their glycogen reserve is very high which is proved by testing with PAS and Best's carmine, with which they give intensely positive reactions (+++). Mercury bromophenol blue also gives an intensely positive reaction (+++) revealing its high protein content. The quantity of fats and bound lipids is rather low, and this is ascertained by very faint positive reactions (+) with Sudan black B and Acetone Sudan black. The cirrus sac gave a very strongly positive reaction (+++) with Indoxyl acetate showing the presence of non-specific esterases and its nervous control (Plate IXa).

The Nervous system : The nervous tissue failed to give any reaction with the conventional methods viz., PAS, Best's carmine, Alcian blue, Mercury bromophenol blue, Sudan black B and Acetone Sudan black. Tests with Indoxyl acetate performed in toto gave a clear picture of the nervous system as well as of other organs rich in non-specific esterases such as the cirrus sac (Plate IXa).

Within the scolex region the above test revealed four ganglions one behind each sucker. These ganglia are interconnected with transverse connections (Plate VIIa,b). The four para-acetabular ganglia give rise to four longitudnal nerves, which extend further

downwards in the neck region and unite to form two longitudnal nerves. From these nerves (in the scolex) arise fine branches which end in the sucker muscles (Plate VIIa,b) thus indicating their nervous control. The two longitudnal nerves take a slight curve in the neck region (Plate VIIIb) with the curve of the post-scolex swelling and further downwards, in the neck region, two branches arise from the two main nerves which first cross each other and later fuse with the subsequent longitudnal nerve (Plate VIIIa). These longitudnal nerves run uniformly parallel through the entire length of the cestode body. In mature seqments fine branches arise from the longitudnal nerves which form a fine network within the segment and every orgen is adequately innervated (Plates IXb,X). In gravid proglottids these fine branches or the network seems to have atrophied but the two longitudnal nerves persist (Plate IXb).

No histochemical work has been done on A.lahorea or its related species neither in sections nor in toto regarding the localization of non-specific esterases. But other cestodes have been studied extensively, notable among these are Tower (1900); Schardein and Waitz (1955); Fripp (1969); Brunkner and Voge (1974) and Krishna et al. (1979).

The above test reveals that Indoxyl acetate is an ideal technique for the staining of nervous tissues which are rich in non-specific esterases.

The Excretory ducts : The excretory ducts failed to give any

reaction with the conventional methods, but Sudan black B gave a slightly positive reaction (+), indicating the presence of fat in them and also suggesting their excretory nature.

DISCUSSION

BIOCHEMICAL STUDIES:

Metabolic studies on helminth parasites were first started by Weinland (1901). Since then much work has been done on these aspects. Some of the notable contributions in this regard are those of Bueding (1950,1953); Von Brand (1950,1952, 1972); Erasmus (1957a, b, 1972); Read (1963) and Smyth (1966). As compared to trematodes much work has been done on cestodes but the studies have been confined to limited groups. In the present study total glycogen, protein and fat were estimated and compared with the results cited by von Brand (1973) on other cestodes. No information relating to such investigations on any species of the genus Avitellina could be traced by the present author, except for the preliminary chrometographic studies by Martinez- Gomez (1966).

The amount of glycogen present in A.lahorea is quite significant. Histochemical observations confirm that most of the glycogen is stored in the cytoplasm of the parenchymal cells and the anastones of the muscles. The total glycogen was found to be 0.981 (±0.01) mg/gm fresh tissue. In M.expansa, Weinland (1901) and Von Brand (1933) have cited glycogen to the extent of 2.7 to 3.2 % of fresh weight and 24-32 % of dry weight. This marked

difference can be easily attributed to the water content of the parasites. Similar difference is found in other cestodes (von Brand, 1973).

The high percentage of protein confirms the histochemical tests for the same. It was found to be 1.40 (± 0.2) mg/gm fresh tissue weight. The protein content of M.expansa is markedly higher. Campbell (1960) has reported 22% protein content in dry tissue. This proportion would certainly be lesser in extent because A.lahorea is comparatively less fleshy than M.expansa. The results could not be confirmed since the total protein has not been worked out in fresh tissue.

In the matter of lipid content, A.lahorea does not offer much contrast against M.expansa; in the former the lipids have been found to range between 2.74-3.78 % (average : 3.396 ± 0.0911%) of fresh weight of tissue whereas in the latter lipids quantify to 3.4% in fresh tissue and 30.1% in dry tissue (von Brand, 1933). This indicates that the lipid uptake gradient is almost consistent in these two anoplocephalids which share the same location in their hosts and that lipids are perhaps not synthesised but are stored of the uptaken fat moieties from the gut contents.

SUMMARY

Standard tests regarding the localization of glycogen, proteins, lipids and acid-mucopolysaccharides viz., PAS, Best's carmine, Alcian blue, Mercury bromophenol blue, Sudan black B and Acetone Sudan black indicated the presence of glycogen in the tegument, the musculature, the parenchyma, the suckers, testes, ovary, uterus and the cirrus sac; of proteins in the tegument, the musculature, the parenchyma, the suckers, testes, ovary, uterus and the cirrus sac and of lipids in the cuticle, the musculature, the parenchyma, the suckers, testes, ovary and the excretory ducts. Alcian blue has also indicated the presence of acid-mucopolysaccharides in the cuticle.

The Indoxyl acetate test performed in toto gave a clear picture of the presence of non-specific esterases. It clearly stained the nervous tissue and, in addition, gave intense reaction in the cirrus sac.

Glycogen, protein and fat contents were quantitatively estimated in the fresh tissue so as to substantiate the histochemical tests. The amount of glycogen was found to be 0.981 ($\pm$0.01) mg/gm; protein 1.40 ($\pm$ 0.02) mg/gm and lipids 2.744 to 3.788 % with an average of 3.306 ($\pm$ 0.0911)%.

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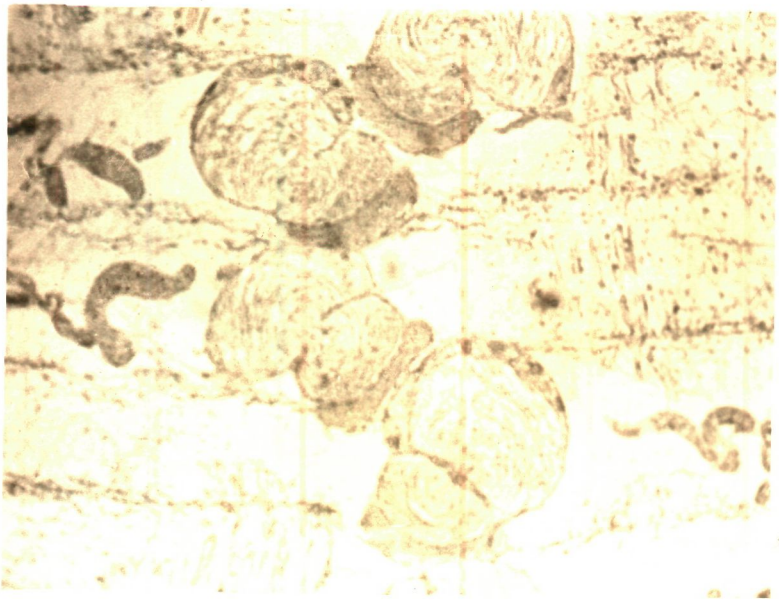
* References not seen in original.

PLATES I - X

PLATE I. a. A.lahorea: Frontal sections of gravid proglottids stained with Haematoxylin and Eosin showing mature paruterine organs, whereas, other sex organs have almost degenerated. The shape of the paruterine organ helps in determining the specific identity of this species.

b. A.lahorea:T.S. of scolex treated with PAS showing the presence of glycogen and other polysaccharides in the suckers, parenchyma and intrinsic muscle fibres.

a



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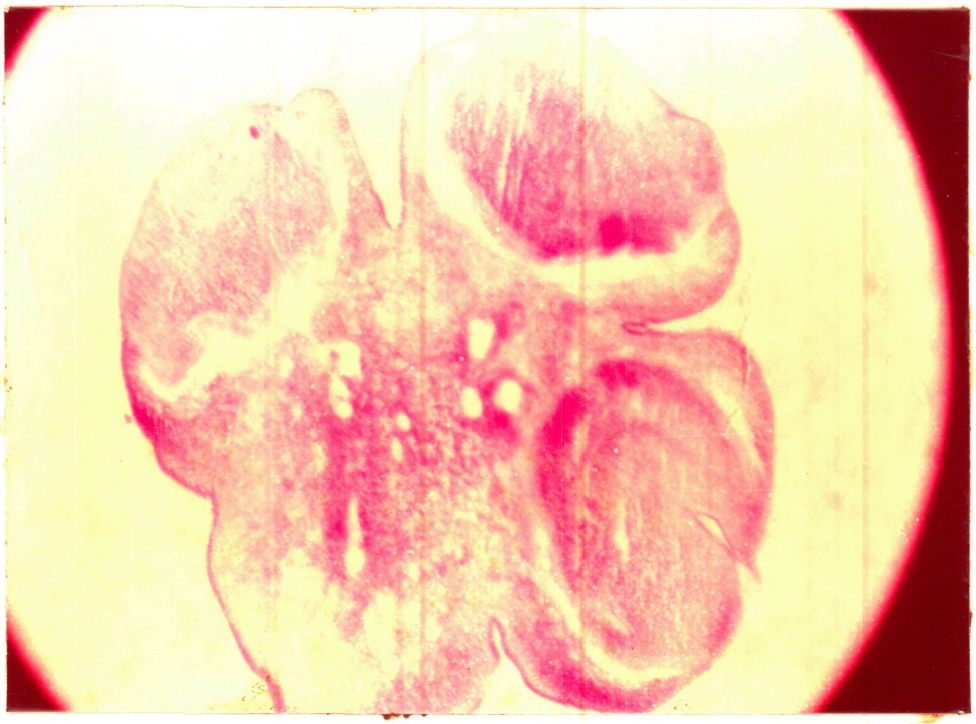
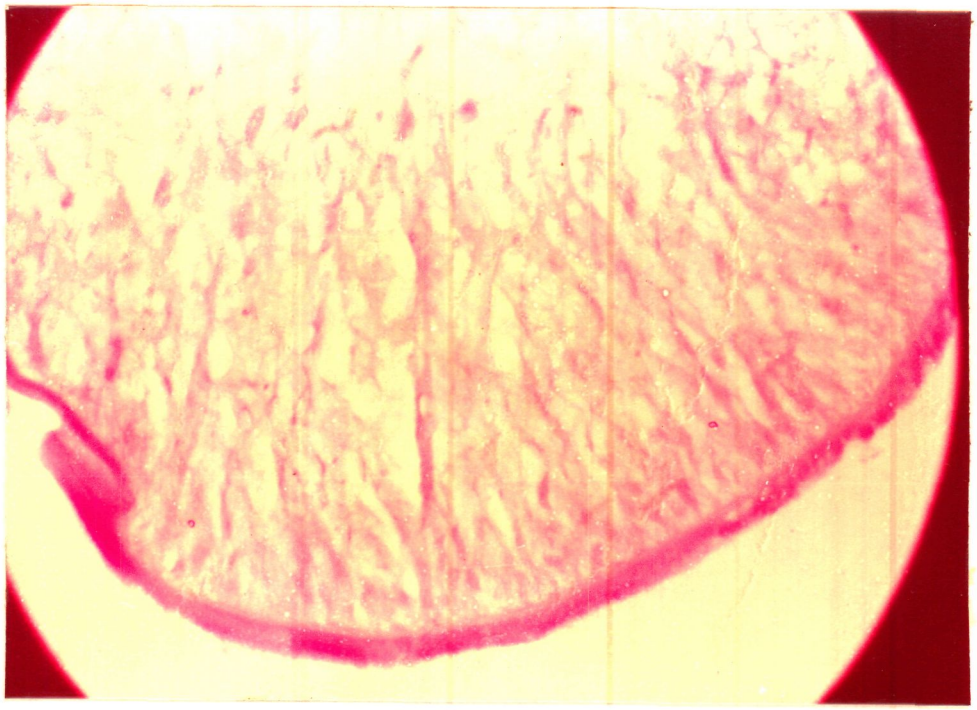


PLATE I

PLATE II. a. A.lahorea: T.S. of immature segment treated with PAS showing the presence of glycogen in the tegument and parenchyma.

b. A.lahorea: T.S. of scolex (acetabular region) treated with PAS showing the presence of glycogen and polysaccharides in the tegument, sucker muscles and the parenchyma.

a



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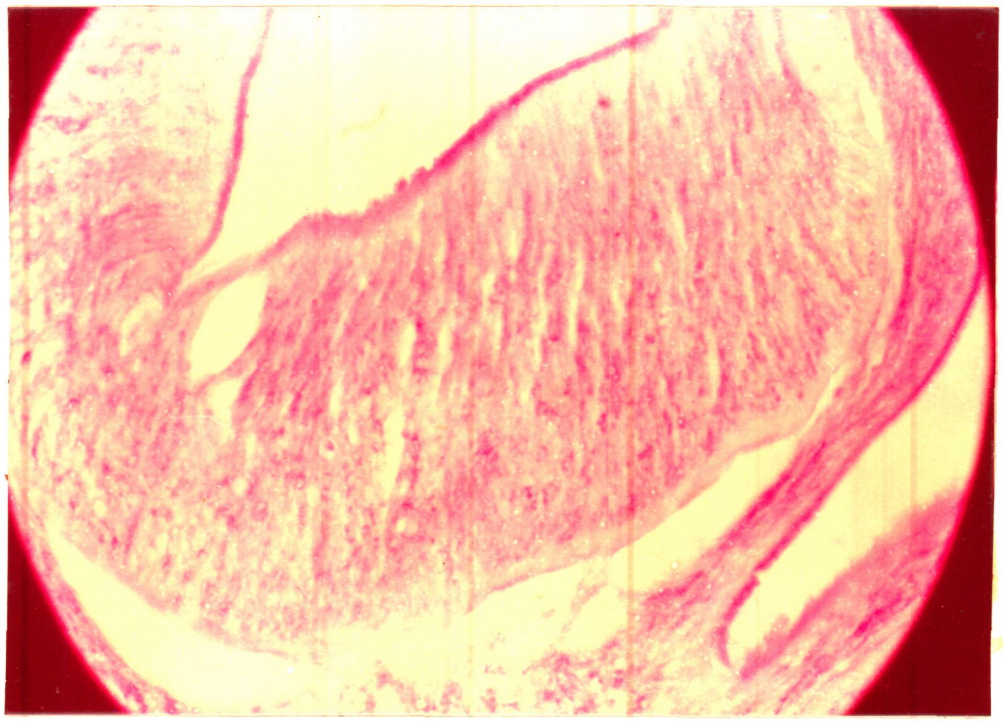
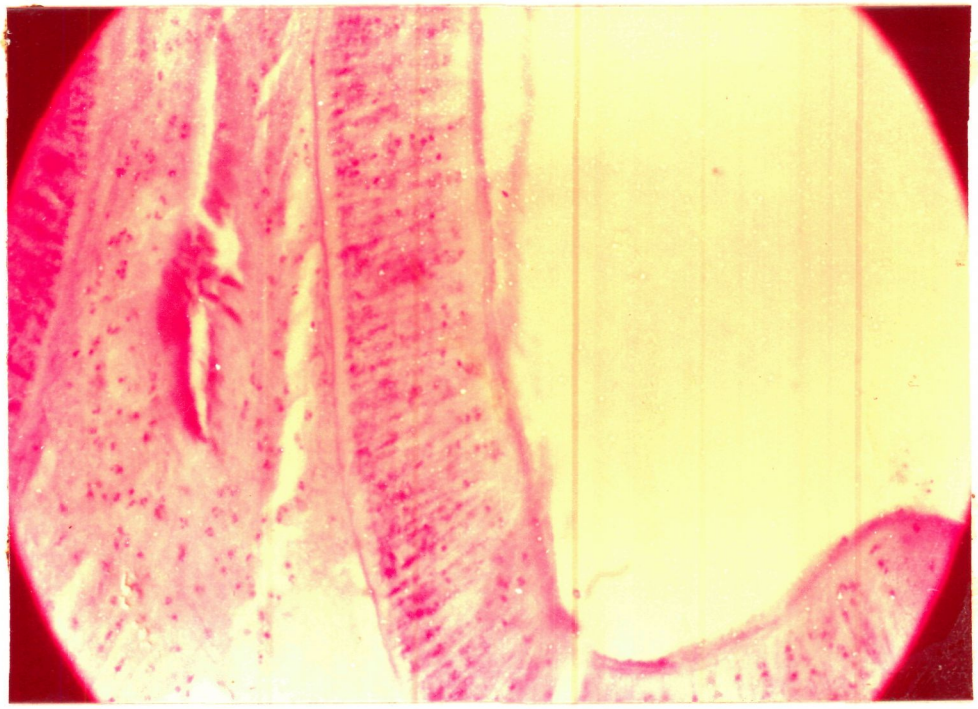


PLATE II

PLATE III. a. A.lahorea: T.S. of scolex (acetabular region) treated with Best's carmine and counter-stained with haematoxylin showing the absence of glycogen in the cuticle and its presence in the subcuticle, sucker muscles and the parenchyma.

b. A.lahorea: T.S. of mature segment treated with Best's carmine showing abundence and rich deposition of glycogen in the subcuticular region and its absence in the tegument.

a



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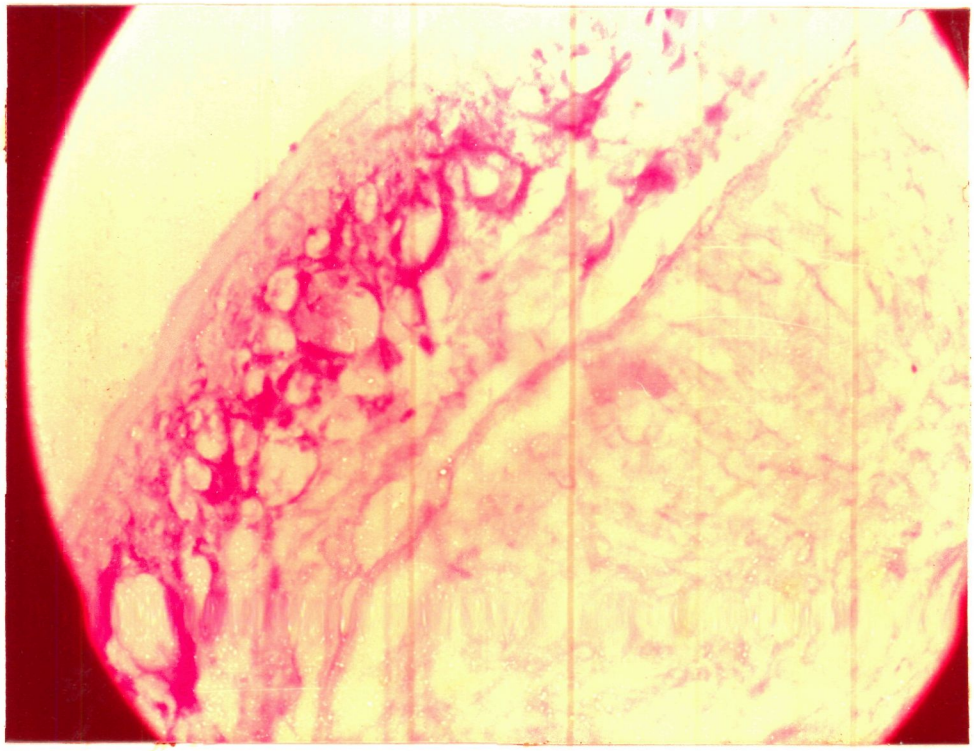


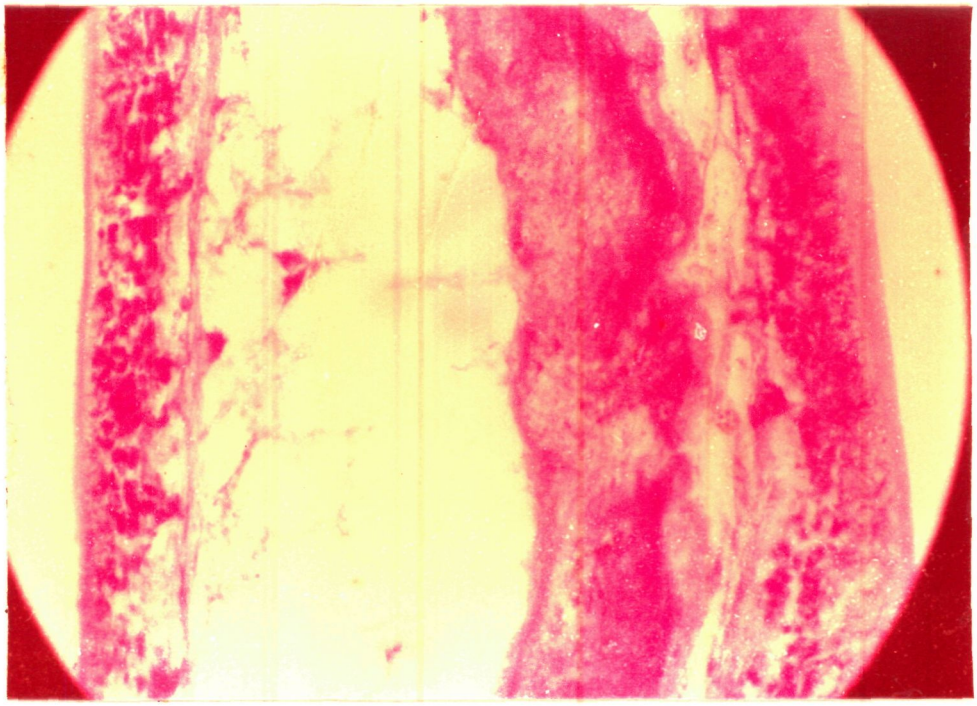
PLATE III

PLATE IV.

a. A.lahorea: L.S. of mature segment treated with PAS showing the presence of glycogen and other polysaccharides in the tegumentary and cortical region indicating high metabolic and glycolytic activity.

b. A.lahorea: T.S. of mature segment partially predigested with α- amylase and then treated with Best's carmine showing the depletion of glycogen to a great extent from the parenchyma indicating that glycogen was markedly present and stored in this region.

a



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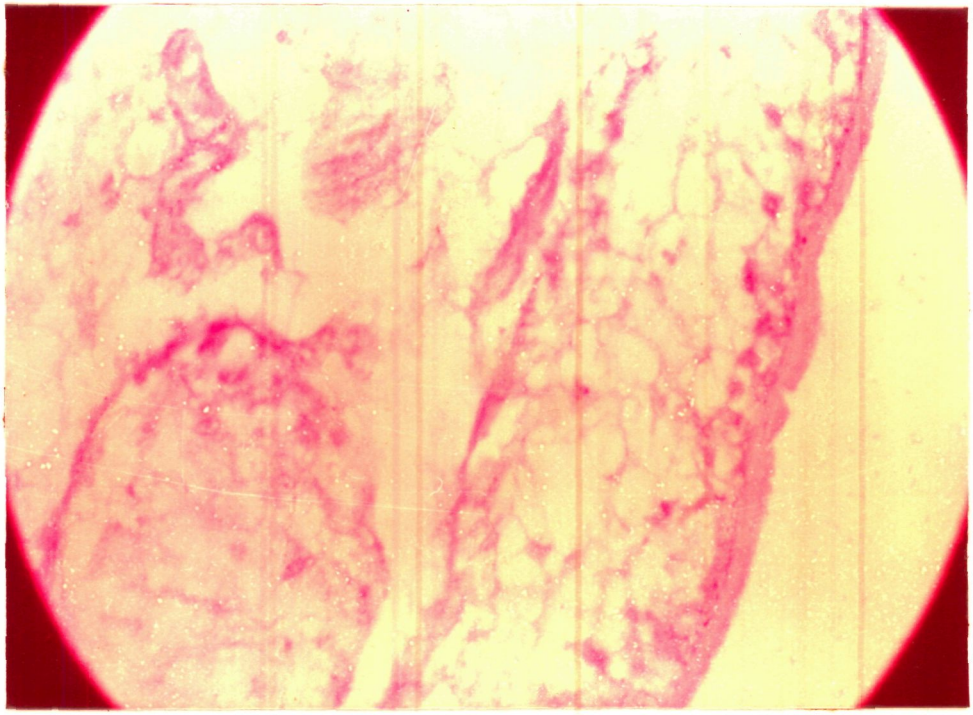


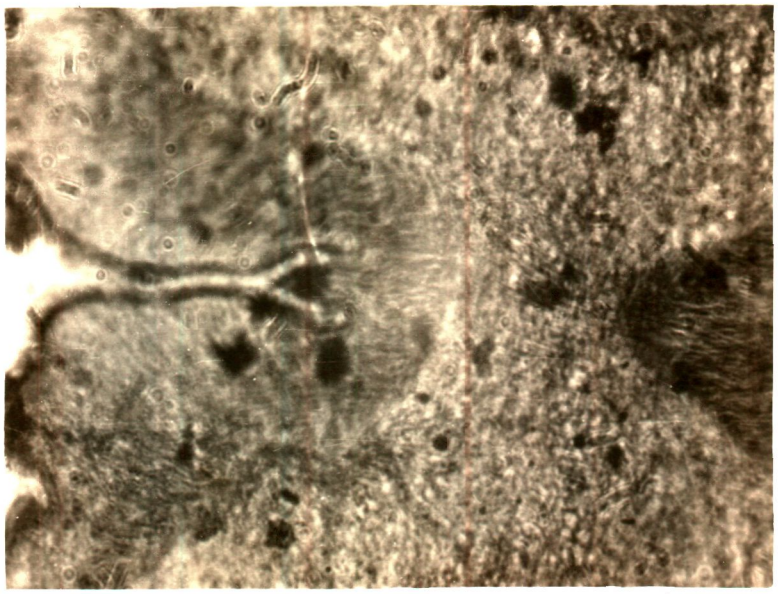
PLATE IV

PLATE V.

a. A.lahorea : T.S. of scolex treated with Acetone Sudan black showing the distribution of bound lipids in the suckers and parenchyma.

b. A.lahorea : T.S. of immature segment treated with Acetone Sudan black showing the deposition of bound lipids in the parenchyma.

a



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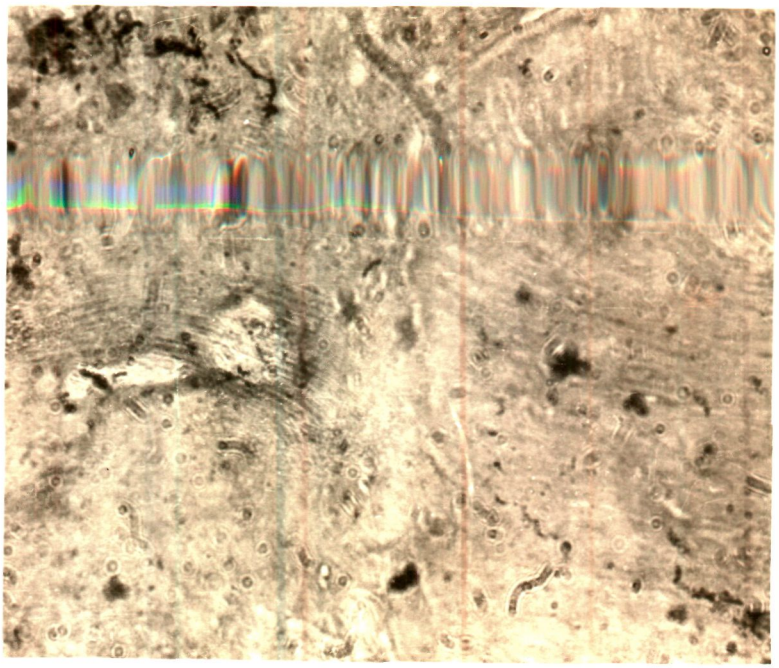


PLATE V

PLATE VI. a. A.lahorea : T.S. of neck region treated with Acetone Sudan black showing the presence of bound lipids in the tegument and parenchyma and their absence around the excretory vessels. The position of dorsal and ventral excretory ducts can also be seen.

b. A.lahorea : T.S. of mature segment treated with Acetone Sudan black showing the deposition of bound lipids in the subcuticle, the parenchyma and the reproductive organs.

a

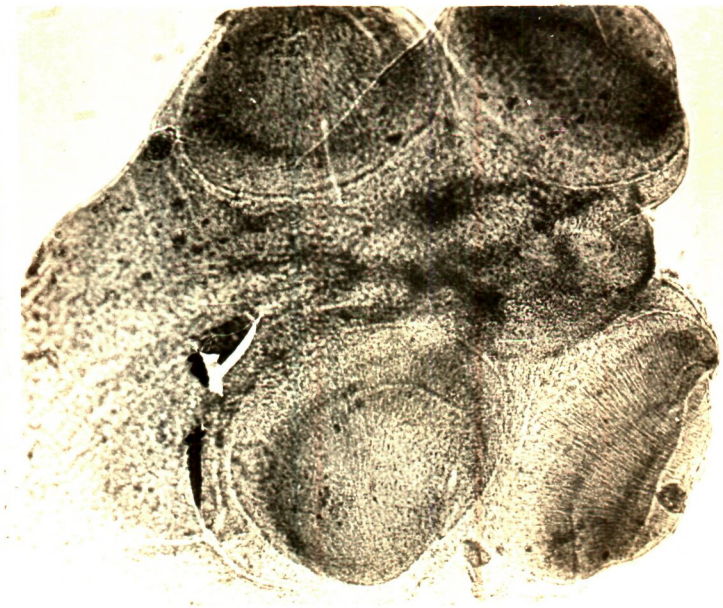
b

PLATE VI

PLATE VII. a. A.lahorea : Toto staining of non-specific esterases in the scolex with Indoxyl acetate.

b. A. lahorea : Scolex stained in toto with Indoxyl acetate showing the four ganglions, their interconnections and also fine nerve endings in the suckers.

a



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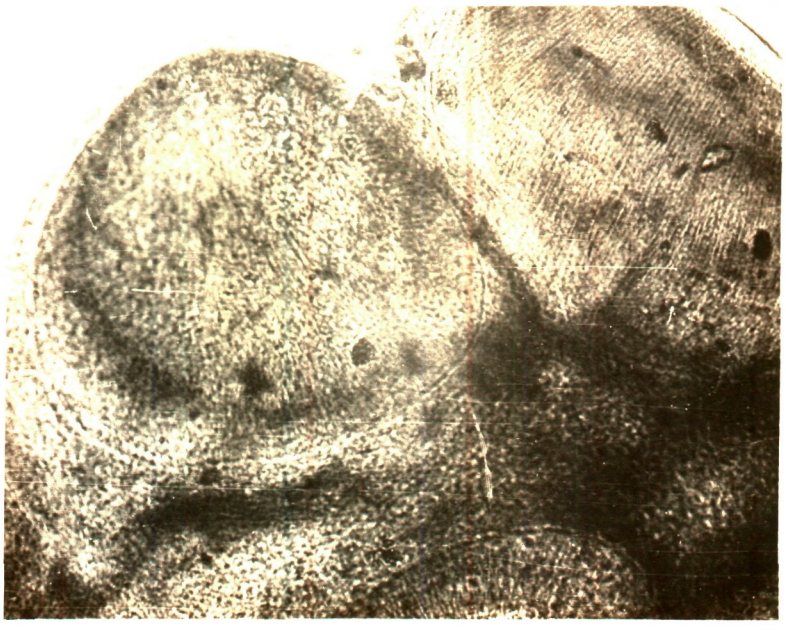


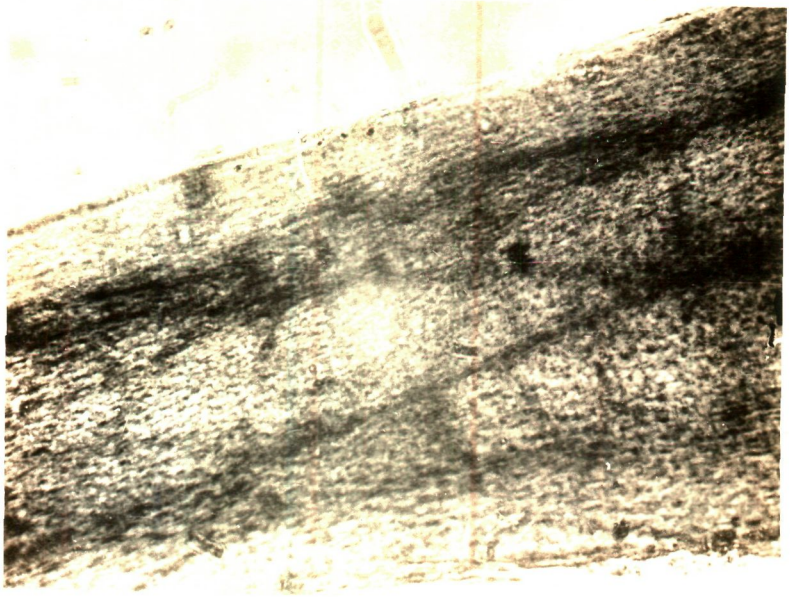
PLATE VII

PLATE VIII.

a. A.lahorea : Immature segments stained with Indoxyl acetate in toto showing the two longitudinal nerves.

b. A.lahorea : Neck region stained with Indoxyl acetate in toto. Note the swelling jus below the suckers and also the curve in the longitudinal nerves with the swelling.

a



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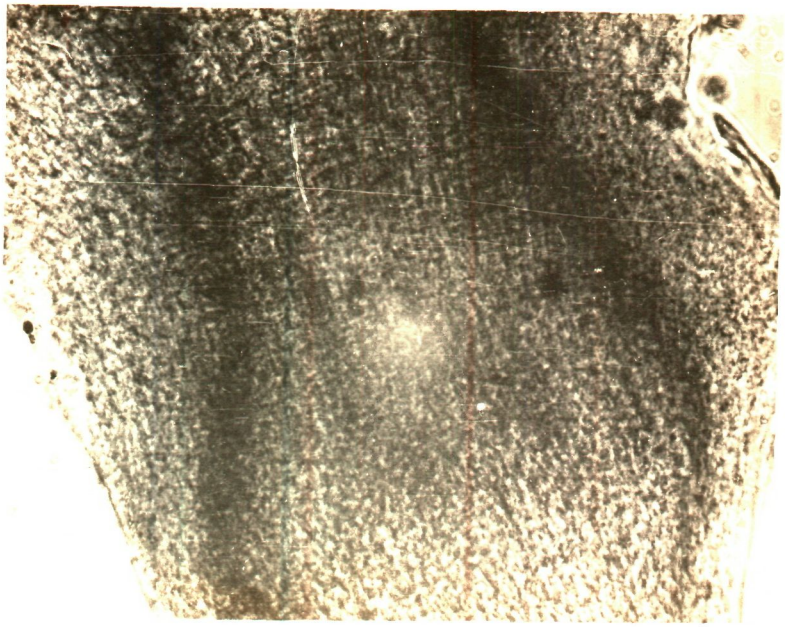


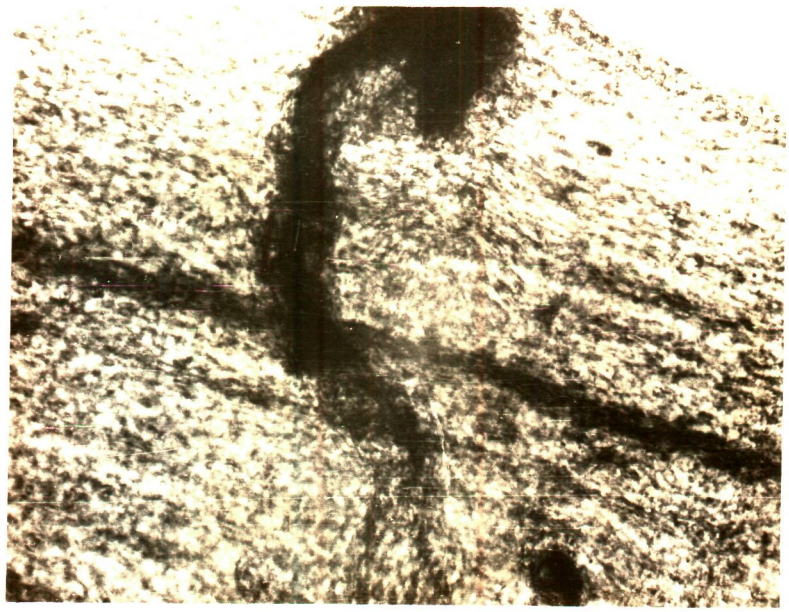
PLATE VIII

PLATE IX.

a. A.lahorea : Mature segment stained in toto with Indoxyl acetate showing the longitudnal nerve and also the presence of esterases(+++) in the cirrus sac.

b. A.lahorea : Gravid proglottids stained in toto with Indoxyl acetate showing the two longitudnal nerve and degenerating transverse nerves. The paruterine organs have started forming the innervation in the mid-proglottidal region seems to undergo atrophy due to the proliferation of the paruterine organs.

a



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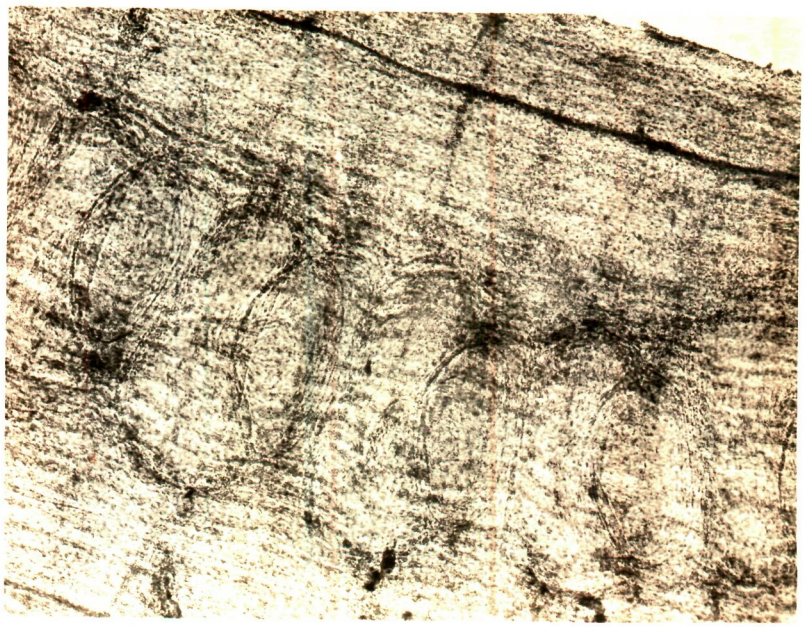

PLATE IX

PLATE X. A.lahorea: Partly gravid segments stained with Indoxyl acetate in toto showing the two longitudnal nerves and fine network of nerves.

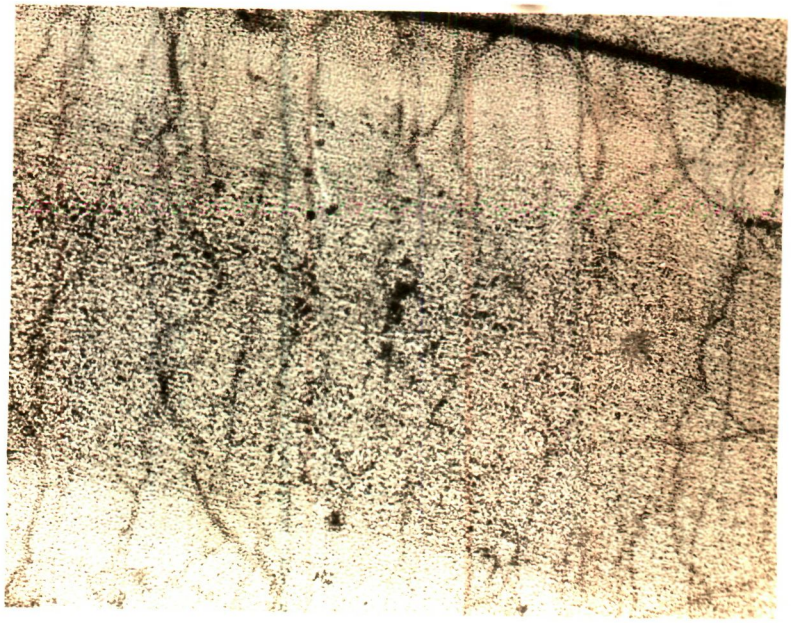

PLATE X